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RNA interference dynamics in juvenile Fasciola hepatica are altered during in vitro growth and development

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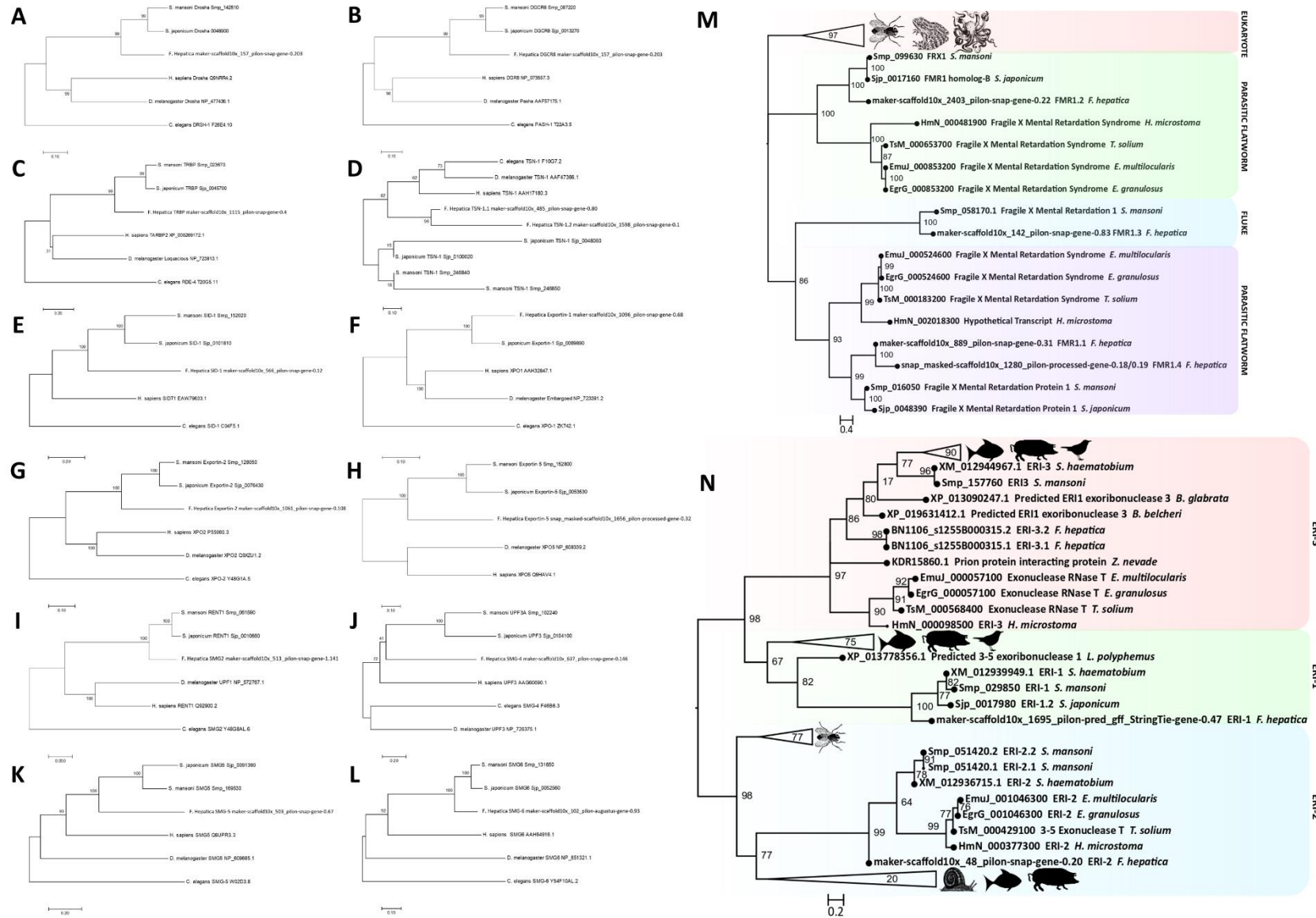
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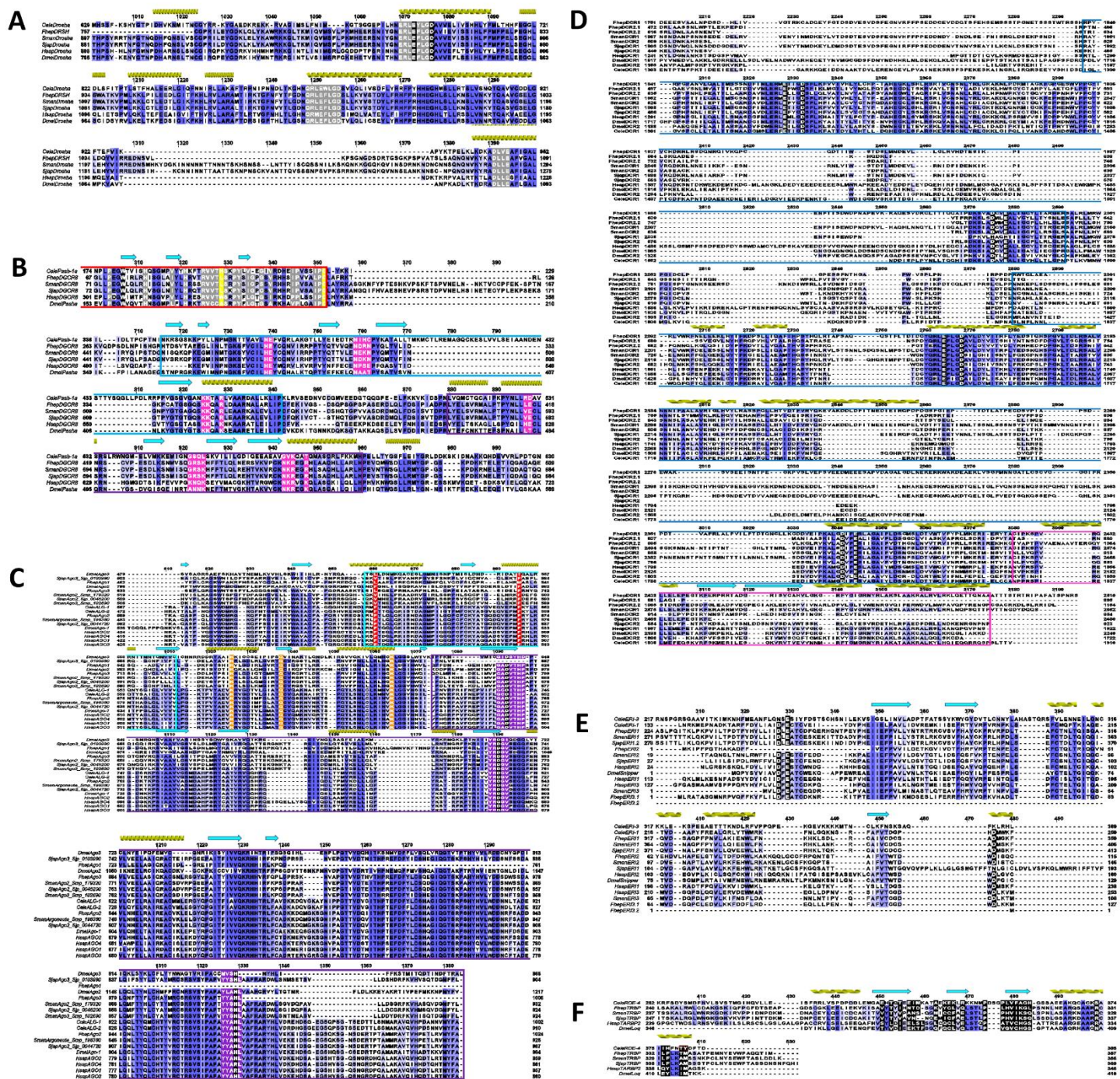
tel: +44 1970 62 2400

email: is@aber.ac.uk

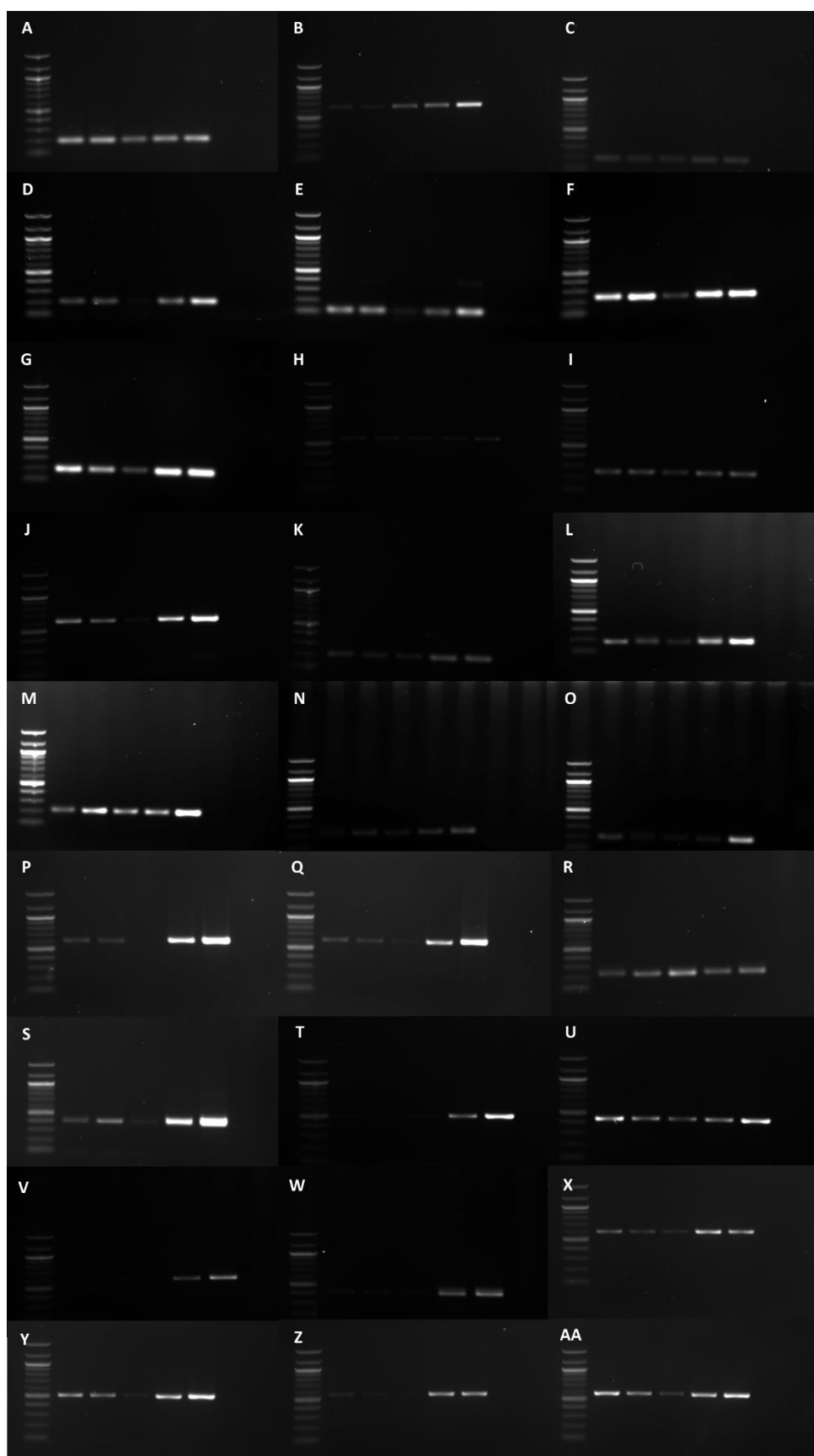
Supplementary Figure 1



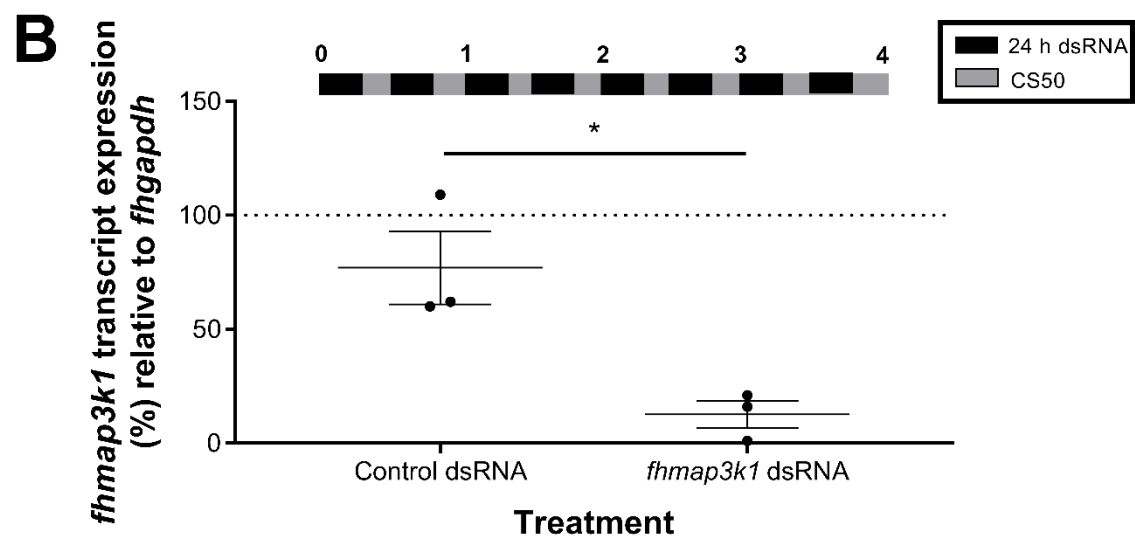
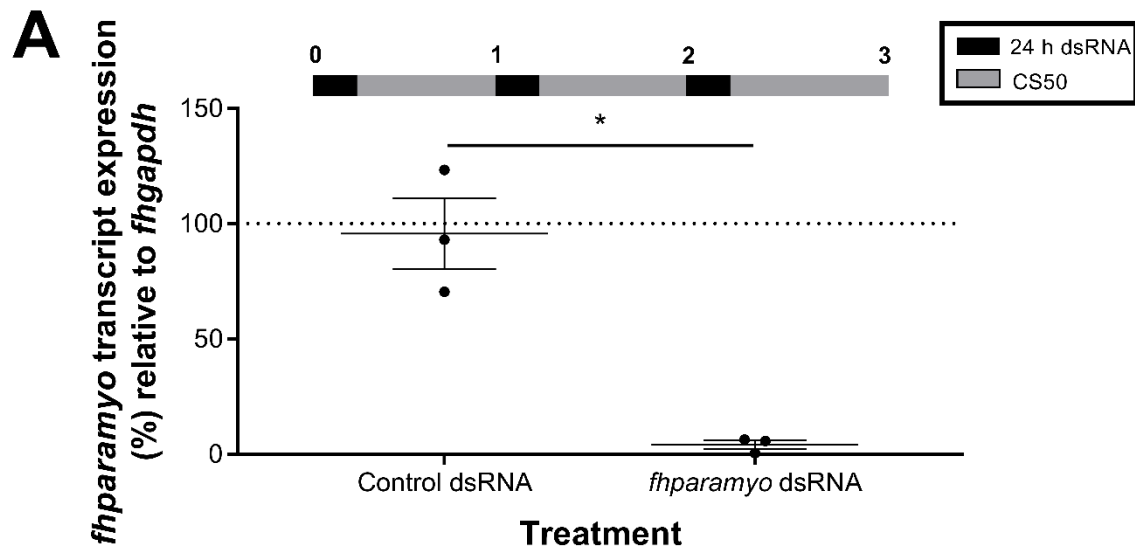
Supplementary Figure 2



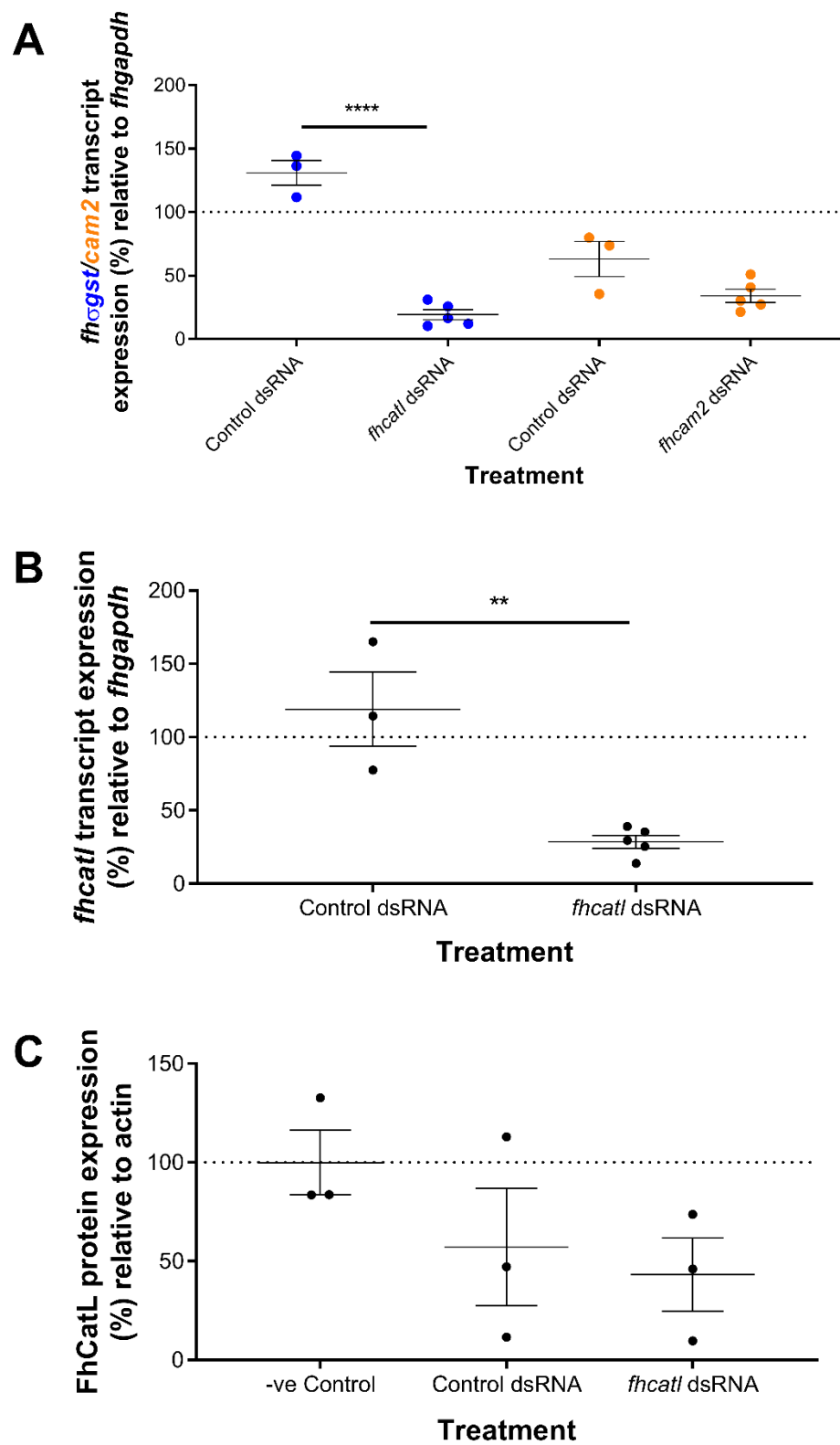
Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5



Supplementary Table 1

Designation	<i>F. hepatica</i> ID (Predicted Protein Dataset)	Forward Primer	Reverse Primer	Product Size
<i>drosha</i>	maker-scaffold10x_157_pilon-snap-gene-0.203	GCAGGCGATTGTGCAGAATC	GCGATCACTGTCACCATTGC	745
<i>dgcr8</i>	maker-scaffold10x_503_pilon-snap-gene-0.75	GGCCATACTCTATCGGTCCA	ATCAGCGGCTGTTAGCAATC	200
<i>dcr-1</i>	maker-scaffold10x_432_pilon-snap-gene-0.93	TTACCGACCGGAAGATGAAC	TCTCCGTTAGTGGGTGTCC	204
<i>dcr-2.1</i>	maker-scaffold10x_1080_pilon-augustus-gene-0.2	ACGCAGTTTTTACCGCCAAC	ACCAACGCTTCCAGCATATC	119
<i>dcr-2.2</i>	maker-scaffold10x_1080_pilon-snap-gene-0.12	CCAAGTGACTGCTGGTTTGC	GGGTGGAACGCCTCACTTA	250
<i>trbp</i>	maker-scaffold10x_1115_pilon-snap-gene-0.4	TTTGCTTGGTCCGATTTC	TGCAGTGGTACTCGTGCATT	197
<i>ago-1</i>	maker-scaffold10x_919_pilon-snap-gene-0.104	GAACTAGCGCATCGAATGGC	CCAGCCAATTCCTCGAGGAG	599
<i>ago-2</i>	maker-scaffold10x_145_pilon-augustus-gene-2.246	AATCAGGCAATTCGATGAG	TGAGAATTCGCGACGGTGA	249
<i>ago-3</i>	maker-scaffold10x_979_pilon-snap-gene-0.4	AATGACTTGCGCCGAATACG	TGTTTTGAGCAGCCCTGTC	578
<i>tsn-1</i>	maker-scaffold10x_485_pilon-snap-gene-0.80	GTTGTCGCATCCGGTATTCT	TGAACCCGTAAAGGGTGAAG	200
	maker-scaffold10x_1598_pilon-snap-gene-0.1	-	-	-
<i>fmr1</i>	maker-scaffold10x_889_pilon-snap-gene-0.31	CCGCGATTCGGTAGAGAAG	ATTCATTCTCGACCACCAC	190
	maker-scaffold10x_2403_pilon-snap-gene-0.22	CATACCGTGCTGTTCCAACC	GCTGATGCGGTTGTTGATAA	211
	maker-scaffold10x_142_pilon-snap-gene-0.83	TACCAGCTGTGTCCTTGTCTG	CTTCAAGAGATCCGGACCAG	216
	snap_masked-scaffold10x_1280_pilon-processed-gene-0.18/0.19	GTCAGACGGGCAGTAATGG	TCGCTGGCTCGACAACTATC	202
<i>sid-1</i>	maker-scaffold10x_566_pilon-snap-gene-0.12	CGGATTTGGGAAGTAATGTGCC	GTACGCCCTCGAGTCTGTGTC	636
<i>exportin-1</i>	maker-scaffold10x_1096_pilon-snap-gene-0.68	GCCCACCGACAAGTTCAAAC	ACACCAGCTTGACGTCGTAG	570
<i>exportin-2</i>	maker-scaffold10x_1061_pilon-snap-gene-0.108	TTCGTCGTATTCTCGCTCG	TTGTTTGGCTCGTCCTCCTC	551
<i>exportin-5</i>	snap_masked-scaffold10x_1656_pilon-processed-gene-0.32	TGCCCTGGTGCCTTTATTAC	ACACGAAACCATTGCACGTA	209
<i>eri1</i>	maker-scaffold10x_1695_pilon-pred_gff_StringTie-gene-0.47	TTGAACCAAGTTGGGCTCTCC	ACAGAATGCAGTCAGGTCCG	501
<i>eri2</i>	maker-scaffold10x_48_pilon-snap-gene-0.20	ACCGCTTTGTGAGGCCTAC	TGTTCTTCGCGTCGTCCATG	441
<i>eri3*</i>	BN1106_s1255B000315.1	TGAGAGCCTCATCTCGAACG	AAGCAATACAATGTTGAGGATG	591
	BN1106_s1255B000315.2	GGATGTCCTGAAGGTGTGTTTTTC	TCCAGTGATTCTCGGGATAGC	410
<i>smg-2</i>	maker-scaffold10x_513_pilon-snap-gene-1.141	GTCCCAGCCCGGATTATCTG	CCATGACTCCTCCGTGATCG	588
<i>smg-4</i>	maker-scaffold10x_637_pilon-snap-gene-0.146	CGAAACCGTCAACACCAACC	ATTGCCGTAGCCTCCATGTC	503
<i>smg-5</i>	maker-scaffold10x_503_pilon-snap-gene-0.67	CGGTCTGAACGCCCTCTATC	TGCGTCCATATCAGGGTTTCG	562
<i>smg-6</i>	maker-scaffold10x_102_pilon-augustus-gene-0.93	GCTCGAGGGTATAGCCACAC	GGCAAACGTGACCAGTTGAC	573

*These predicted genes are absent from new genome assembly, however PCR data have confirmed their presence and expression in juvenile and adult worms

Supplementary Table 2

Designation	ID	NCBI/Wormbase Parasite ID	Forward dsRNA Primer*	Reverse dsRNA Primer*	Forward qPCR Primer	Reverse qPCR Primer
<i>fhcam2</i>	Calmodulin 2	AM412547/maker-scaffold10x_520_pilon-snap-gene-1.36	AGATGCCACGGAAGTAAAAG	AAATGTACCCGTCGCCATTA	ATCGCGGCAATCTTGATAAT	TTGCGCAGAAGAGTCAAGAA
<i>fhgst</i>	σ Glutathione s-transferase	DQ974116/maker-scaffold10x_1043_pilon-snap-gene-0.18	AATTCGCCTTCTGCTCACTTGC	TACTCTTCGTCTGTTTCACC	AATTCGCCTTCTGCTCACTTGC	TCTTCACACTACCAATGATACG
<i>fhparamyo</i>	Paramyosin	augustus_masked-scaffold10x_377_pilon-processed-gene-0.188	CTCTGGCATCTGCACGTAAA	TTCCTCGATCTGCTGTTGTG	TTGAAATCCGCGAAATTACC	ATCGACGTGATTACCATCA
<i>fhmap3k1</i>	Mitogen Activated Protein 3k1	maker-scaffold10x_208_pilon-snap-gene-0.24	AGCCCCACACAATACCAGAG	AATTGGTGGAAGTGGTGG	AGCCCCACACAATACCAGAG	TGTACCTGCTAAGCGTCGTG
<i>fhcatl</i>	Cathepsin L	See McVeigh et al. 2014	TKRTTATGTGACGGAGGTGA	GCCBKRTAHGGRTAAK	TKRTTATGTGACGGAGGTGA	GTATAGAAGCCAGTCACTTTGGC
<i>fhgapdh</i>	GAPDH	AY005475/maker-scaffold10x_2706_pilon-snap-gene-0.15			GGCTGTGGGCAAAGTCATTC	AGATCCACGACGGAAACATCA
<i>neo</i>	Bacterial Neomycin	U55762	GGTGGAGAGGCTATTTCGGCT	CCTTCCCGTTTCAGTGACAA		

*dsRNA constructs were amplified with primers labelled at the 5' end with T7 RNA polymerase promoter sequence (taatacgactcactatagggt) to make T7 labelled templates in each direction

Supplementary Table 3

Name	S. mansoni ID	F. hepatica ID	Length (aa)	% Identity with <i>S. mansoni</i>	Domains/Pfam IDs	Signature Motifs Identified	
Drosha	Smp_142510 (1531aa)	maker-scaffold10x_157_pilon-snap-gene-0.203	1640	45.00	Ribonuclease III domain (IPR000999)	Endonuclease 1 ERLEFLGD	Endonuclease 2 QRLEFLGD... DLLE
					Double-stranded RNA-binding domain (IPR014720)		
DGCR8	Smp_087220 (761aa)	maker-scaffold10x_503_pilon-snap-gene-0.75	457	37.60	Double-stranded RNA-binding domain (IPR014720)		
Dicer-1	Smp_169750 (2646aa)	maker-scaffold10x_432_pilon-snap-gene-0.93	2422	46.10	P-loop containing nucleoside triphosphate hydrolase (IPR027417)	3' End Pocket SPFPTSTY...NFAAYYM SKY...IPELC	5' End Pocket RGRPCYRPEDEPLGFLTTKPLHHI PTFPFISRSGEETVRF...TVMRLSLIVP RYVN
					Dicer dimerisation domain (IPR005034)		
					PAZ domain (IPR003100)		
					Ribonuclease III domain (IPR000999)		
Dicer-2	Smp_033600 (954aa)	maker-scaffold10x_1080_pilon-augustus-gene-0.2	601	30.83	Double-stranded RNA-binding domain (IPR014720)		
					Ribonuclease III domain (IPR000999)	3' End Pocket DPVPTKLS...SYVDYFSI RY...LADTC	3' End Pocket RHHEDFDPGKLTDSRSNIVSNNSLAS AVVEHKIHPY(2088)...GACRKDLSLR RID
		maker-scaffold10x_1080_pilon-snap-gene-0.12	1091	41.50	Ribonuclease III domain (IPR000999)	3' End Pocket DPVPTKLS...SYVDYFSI RY...LADTC	3' End Pocket RHHEDFDPGKLTDSRSNIVSNNSLAS AVVEHKIHPY(2088)...GACRKDLSLR RID
TRBP	Smp_023670 (356aa)	maker-scaffold10x_1115_pilon-snap-gene-0.4	357	56.10	Double-stranded RNA-binding domain (IPR014720),		
Argonaute-1	Smp_198380 (928aa)	maker-scaffold10x_919_pilon-snap-gene-0.104	675	17.00	Argonaute, linker 1 domain (IPR014811)	Piwi Magnesium Ion Interacting Residues GADVTHP...YRDGV	
					PAZ domain (IPR003100)		
					Ribonuclease H-like domain (IPR012337)		
					Piwi domain (IPR003165)		
Argonaute-2	Smp_179320 (932aa)	maker-scaffold10x_145_pilon-augustus-gene-2.246 (BN1106_s1192B000304)	355 (1024)	16.8 (28.3)	Protein argonaute, N-terminal (IPR032474)	Piwi Magnesium Ion Interacting Residues GADVTHP...YRDGV...Y Y AHL	
					Argonaute, linker 1 domain (IPR014811)		
					PAZ domain (IPR003100)		
					Argonaute linker 2 domain (IPR032472)		
					Protein argonaute, Mid domain (IPR032473)		
					Ribonuclease H-like domain (IPR012337)-		
Argonaute-3	Smp_102690 (854aa)	maker-scaffold10x_979_pilon-snap-gene-0.4	898	36.60	Piwi domain (IPR003165)	Piwi Magnesium Ion Interacting Residues GADVTHP...YRDGV...Y YSHL	
					Argonaute, linker 1 domain (IPR014811), Ribonuclease H-like domain (IPR012337)		
Tudor-SN	Smp_246840 (992aa)	maker-scaffold10x_485_pilon-snap-gene-0.80	1098	50.40	Staphylococcal nuclease (SNase-like), OB-fold (IPR016071),		
	Smp_246850 (378aa)	maker-scaffold10x_1598_pilon-snap-gene-0.1 (BN1106_s375B000233)	1220 (518)	8.0 (22.22)	Staphylococcal nuclease (SNase-like), OB-fold (IPR016071), Tudor domain (IPR002999)		
FMR1	Smp_099630 (598aa)	BN1106_s2932B000172	690	20.40	K Homology domain (IPR004087)		
					K Homology domain, type 1 (IPR004088)		
		maker-scaffold10x_2403_pilon-snap-gene-0.22 (BN1106_s6067B000089)	1015 (460)	24.1 (43.7)	K Homology domain, type 1 (IPR004088)		
	Smp_058170 (827aa)	maker-scaffold10x_142_pilon-snap-gene-0.83	812	44.70	K Homology domain, type 1 (IPR004088)		

		snap_masked-scaffold10x_1280_pilon-processed-gene-0.18/snap_masked-scaffold10x_1280_pilon-processed-gene-0.19	569	15.50	K Homology domain, type 1 (IPR004088)		
SID-1	Smp_152020 (933aa)	maker-scaffold10x_566_pilon-snap-gene-0.12	864	44.80	PF13965 (SID-1_RNA_chan)		
Exportin-1	Smp_124820 (1075aa)	maker-scaffold10x_1096_pilon-snap-gene-0.68	1211	72.30	Armadillo-type fold (IPR016024) Armadillo-like helical (IPR011989) Importin-beta, N-terminal domain (IPR001494) Exportin-1/Importin-beta-like (IPR013598) CRM1 C-terminal domain (IPR014877)		
Exportin-2	Smp_128050 (1049aa)	maker-scaffold10x_1061_pilon-snap-gene-0.108	1061	60.00	Armadillo-type fold (IPR016024) Armadillo-like helical (IPR011989) Importin-beta, N-terminal domain (IPR001494) Exportin/Importin, Cse1-like (IPR013713) Exportin/Importin, Cse1-like (IPR013713) CAS/CSE, C-terminal (IPR005043)		
Exportin-5	Smp_152800 (1286aa)	snap_masked-scaffold10x_1656_pilon-processed-gene-0.32	786	25.10	Armadillo-type fold (IPR016024) Armadillo-like helical (IPR011989)		
ERI1	Smp_029850 (562aa)	maker-scaffold10x_1695_pilon-pred_gff_StringTie-gene-0.47	788	38.70	Ribonuclease H-like domain (IPR012337) Exonuclease, RNase T/DNA polymerase III (IPR013520)	Nuclease D...E...D...H...D	
ERI2	Smp_051420 (232aa)	maker-scaffold10x_48_pilon-snap-gene-0.20	240	57.45	Ribonuclease H-like domain (IPR012337) Exonuclease, RNase T/DNA polymerase III (IPR013520)	Nuclease D...H...D	
ERI3*	Smp_157760 (191aa)	BN1106_s1255B000315.1	211	54.30	Ribonuclease H-like domain (IPR012337) Exonuclease, RNase T/DNA polymerase III (IPR013520)	Nuclease D...E...D...H...D	
		BN1106_s1255B000315.2	85	55.95	Ribonuclease H-like domain (IPR012337) Exonuclease, RNase T/DNA polymerase III (IPR013520)	Nuclease H...D	
SMG2	Smp_061590 (1325aa)	maker-scaffold10x_513_pilon-snap-gene-1.141	1336	74.10	RNA helicase UPF1, UPF2-interacting domain (IPR018999) P-loop containing nucleoside triphosphate hydrolase (IPR027417)		
SMG4	Smp_102240 (560aa)	maker-scaffold10x_637_pilon-snap-gene-0.146	456	12.90	Regulator of nonsense-mediated decay, UPF3 (IPR005120) Nucleotide-binding alpha-beta plait domain (IPR012677)		
SMG5	Smp_169530 (1479aa)	maker-scaffold10x_503_pilon-snap-gene-0.67	1658	31.50	Tetratricopeptide-like helical domain (IPR011990) DNA/RNA-binding domain, Est1-type (IPR018834)		
SMG6	Smp_131650 (1409aa)	maker-scaffold10x_102_pilon-augustus-gene-0.93 (BN1106_s2800B000128)	224 (1381)	3.3 (54.2)	Tetratricopeptide-like helical domain (IPR011990) Telomerase activating protein Est1 (IPR019458) DNA/RNA-binding domain, Est1-type (IPR018834) PIN domain-like (IPR029060) PIN domain (IPR002716)		

*These predicted genes are absent from new genome assembly, however PCR data have confirmed their presence and expression in juvenile and adult worms

Supplementary File 1

Droscha Sequences

Q9NRR4.2 (NCBI)
XP_010815134.1 (NCBI)
NP_477436.1 (NCBI)
CE29039 (Wormbase)
BM25607 (Wormbase)
CN03400 (Wormbase)
CBP41277 (Wormbase)
JA61114 (Wormbase)
RPA2472 (Wormbase)
OVP08524 (Wormbase)
Sjp_0048900 (Wormbase Parasite)
Smp_142510 (Wormbase Parasite)
Ancylostoma caninum (Dalzell et. al. 2011)
Ascaris suum (Dalzell et. al. 2011)
Meloidogyne hapla (Dalzell et. al. 2011)
Meloidogyne incognita (Dalzell et. al. 2011)
Pristionchus pacificus (Dalzell et. al. 2011)
Trichinella spiralis (Dalzell et. al. 2011)

DGCR8 Sequences

NP_073557.3 (NCBI)
NP_001094674.1 (NCBI)
NP_651879.1 (NCBI)
Smp_087220 (Wormbase Parasite)
Sjp_0013270 (Wormbase Parasite)
CE48538 PASH-1 (Wormbase)
BM38243 PASH-1 (Wormbase)
CBP43006 PASH-1 (Wormbase)
JA65435 PASH-1 (Wormbase)
RP39638 PASH-1 (Wormbase)
OVP13372 PASH-1 (Wormbase)
PP03672 PASH-1 (Wormbase)
Caenorhabditis brenneri (Dalzell et. al. 2011)
Haemonchus contortus (Dalzell et. al. 2011)
Meloidogyne hapla (Dalzell et. al. 2011)
Meloidogyne incognita (Dalzell et. al. 2011)

Dicer Sequences

NP_001278557.1 (NCBI)
AAR26432.1 (NCBI)
NP_52453.1 (NCBI)
NP_523778.2 (NCBI)
ECK26346.1 (NCBI)
Smp_169750 (Wormbase Parasite)
Smp_053600 (Wormbase Parasite)
Sjp_0069770 (Wormbase Parasite)
Sjp_0043700 (Wormbase Parasite)
CE47418 DCR-1 (Wormbase)
BM36861 DCR-1 (Wormbase)
CN04613 DCR-1 (Wormbase)
CBP41384 DCR-1 (Wormbase)
JA44861 DCR-1 (Wormbase)
OVP14022 DCR-1 (Wormbase)
Ancylostoma caninum (Dalzell et. al. 2011)
Ascaris suum (Dalzell et. al. 2011)
Meloidogyne hapla (Dalzell et. al. 2011)
Meloidogyne incognita (Dalzell et. al. 2011)
Oesophagostomum dentatum (Dalzell et. al. 2011)
Pristionchus pacificus (Dalzell et. al. 2011)
Trichinella spiralis (Dalzell et. al. 2011)

DRH-1 Sequences

NP_055129.2 (NCBI)
NP_002689526.3 (NCBI)
EKC38303.1 (NCBI)
CE16988 (Wormbase)
BM40905 (Wormbase)
CN02053 (Wormbase)
CBP38705 (Wormbase)
JA04049 (Wormbase)
RPA1755 (Wormbase)
Ancylostoma caninum (Dalzell et. al. 2011)
Ascaris suum (Dalzell et. al. 2011)
Haemonchus contortus (Dalzell et. al. 2011)
Meloidogyne hapla (Dalzell et. al. 2011)
Meloidogyne incognita (Dalzell et. al. 2011)
Trichinella spiralis (Dalzell et. al. 2011)

DRH-3 Sequences

CE36120 (Wormbase)
BM45565 (Wormbase)
CBP46809 (Wormbase)
JA52630 (Wormbase)
RPA48730 (Wormbase)
OVP14314 (Wormbase)
PP30574 (Wormbase)
Ancylostoma caninum (Dalzell et. al. 2011)
Ascaris suum (Dalzell et. al. 2011)
Caenorhabditis brenneri (Dalzell et. al. 2011)
Haemonchus contortus (Dalzell et. al. 2011)
Meloidogyne hapla (Dalzell et. al. 2011)
Meloidogyne incognita (Dalzell et. al. 2011)
Oesophagostomum dentatum (Dalzell et. al. 2011)
Trichinella spiralis (Dalzell et. al. 2011)

RDE-4 Sequences

CE00630 (Wormbase)
BM23272 (Wormbase)
CBP08423 (Wormbase)
JA48137 (Wormbase)
RPA14524 (Wormbase)
PP50570 (Wormbase)
Ancylostoma caninum (Dalzell et. al. 2011)
Caenorhabditis brenneri (Dalzell et. al. 2011)

RRF-1 Sequences

CE27141 (Wormbase)
CBP37456 (Wormbase)
RPA6078 (Wormbase)
Ascaris suum (Dalzell et. al. 2011)
Caenorhabditis brenneri (Dalzell et. al. 2011)

Argonaute-like Sequences

NP_036331.1 (NCBI)
NP_036286.2 (NCBI)
NP_079128.2 (NCBI)
NP_060099.2 (NCBI)
NP_001192628.1 (NCBI)
AA521301.1 (NCBI)
AAR12162.2 (NCBI)
NP_725341.1 (NCBI)
NP_648775.1 (NCBI)
EKC35067.1 (NCBI)
Smp_102690 (Wormbase Parasite)
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Smp_179320 (Wormbase Parasite)
Sjp_0044720 (Wormbase Parasite)
Sjp_0045200 (Wormbase Parasite)
Sjp_0103990 (Wormbase Parasite)
CE11525 (Wormbase)
CE32063 (Wormbase)
CE18277 (Wormbase)
CE01117 (Wormbase)
CN03079 (Wormbase)
CBP07776 (Wormbase)
CBP40745 (Wormbase)
JA63108 (Wormbase)
RP37764 (Wormbase)

PP22070 (Wormbase)
Ancylostoma caninum (Dalzell et. al. 2011)
Ascaris suum (Dalzell et. al. 2011)
Brugia malayi (Dalzell et. al. 2011)
Haemonchus contortus (Dalzell et. al. 2011)
Meloidogyne hapla (Dalzell et. al. 2011)
Meloidogyne incognita (Dalzell et. al. 2011)
Oesophagostomum dentatum (Dalzell et. al. 2011)
Pristionchus pacificus (Dalzell et. al. 2011)
Trichinella spiralis (Dalzell et. al. 2011)
Ascaris suum (Dalzell et. al. 2011)
Haemonchus contortus (Dalzell et. al. 2011)
Meloidogyne hapla (Dalzell et. al. 2011)
Meloidogyne incognita (Dalzell et. al. 2011)
Trichinella spiralis (Dalzell et. al. 2011)

PIWI-Clade AGO Sequences

AAC93731.2 (NCBI)
BAC81342.1 (NCBI)
BAC81343.1 (NCBI)
NP_689644.2 (NCBI)
NP_001302055.1 (NCBI)
NP_015320079.1 (NCBI)
NP_001306825.1 (NCBI)
NP_015321929.1 (NCBI)
AGL81535.1 (NCBI)
CAAGA320.1 (NCBI)
EKC35279.1 (NCBI)
EKC29295.1 (NCBI)
CE30106 ERGO-1 (Wormbase)
CBP27166 ERGO-1 (Wormbase)
JA40280 ERGO-1 (Wormbase)
RP28315 ERGO-1 (Wormbase)
OVP00965 ERGO-1 (Wormbase)
Caenorhabditis brenneri (Dalzell et. al. 2011)
Trichinella spiralis (Dalzell et. al. 2011)
CE09083 (Wormbase)
CE06748 (Wormbase)
CBP09000 (Wormbase)
Ancylostoma caninum (Dalzell et. al. 2011)
Caenorhabditis brenneri (Dalzell et. al. 2011)
Caenorhabditis japonica (Dalzell et. al. 2011)
Pristionchus pacificus (Dalzell et. al. 2011)
Haemonchus contortus (Dalzell et. al. 2011)
Oesophagostomum dentatum (Dalzell et. al. 2011)

Helminth-only AGO

Sequences

CE06244 (Wormbase)
CE17921 (Wormbase)
CE48028 (Wormbase)
CE20101 (Wormbase)
CE22246 (Wormbase)
CN20858 (Wormbase)
CN25357 (Wormbase)
CN21626 (Wormbase)
JA14615 (Wormbase)
RP04583 (Wormbase)
PP44473 (Wormbase)
Ancylostoma caninum (Dalzell et. al. 2011)
Ascaris suum (Dalzell et. al. 2011)
Brugia malayi (Dalzell et. al. 2011)
Haemonchus contortus (Dalzell et. al. 2011)
Meloidogyne hapla (Dalzell et. al. 2011)
Meloidogyne incognita (Dalzell et. al. 2011)
Oesophagostomum dentatum (Dalzell et. al. 2011)
Pristionchus pacificus (Dalzell et. al. 2011)

Ancylostoma caninum (Dalzell et. al. 2011)
Ascaris suum (Dalzell et. al. 2011)
Brugia malayi (Dalzell et. al. 2011)
Haemonchus contortus (Dalzell et. al. 2011)
Meloidogyne hapla (Dalzell et. al. 2011)
Meloidogyne incognita (Dalzell et. al. 2011)
Oesophagostomum dentatum (Dalzell et. al. 2011)
Pristionchus pacificus (Dalzell et. al. 2011)
Ancylostoma caninum (Dalzell et. al. 2011)
Ascaris suum (Dalzell et. al. 2011)
Brugia malayi (Dalzell et. al. 2011)
Haemonchus contortus (Dalzell et. al. 2011)
Meloidogyne hapla (Dalzell et. al. 2011)
Meloidogyne incognita (Dalzell et. al. 2011)
Oesophagostomum dentatum (Dalzell et. al. 2011)
Ancylostoma caninum (Dalzell et. al. 2011)

Ascaris suum (Dalzell et. al. 2011)
Haemonchus contortus (Dalzell et. al. 2011)
Oesophagostomum dentatum (Dalzell et. al. 2011)
Ancylostoma caninum (Dalzell et. al. 2011)
Meloidogyne incognita (Dalzell et. al. 2011)
Oesophagostomum dentatum (Dalzell et. al. 2011)
Pristionchus pacificus (Dalzell et. al. 2011)
CE12164 (Wormbase)
CE25906 (Wormbase)
CBP37437 (Wormbase)
CBP39116 (Wormbase)
JA07867 (Wormbase)
RP46812 (Wormbase)

Ancylostoma caninum (Dalzell et. al. 2011)
Ascaris suum (Dalzell et. al. 2011)
Caenorhabditis brenneri (Dalzell et. al. 2011)
Haemonchus contortus (Dalzell et. al. 2011)
Meloidogyne hapla (Dalzell et. al. 2011)
Meloidogyne incognita (Dalzell et. al. 2011)
Oesophagostomum dentatum (Dalzell et. al. 2011)
Trichinella spiralis (Dalzell et. al. 2011)
CE28243 (Wormbase)
BM38004 (Wormbase)
CN02916 (Wormbase)
CBP65899 (Wormbase)
JA45228 (Wormbase)
RP45359 (Wormbase)
Ancylostoma caninum (Dalzell et. al. 2011)
Ascaris suum (Dalzell et. al. 2011)
Haemonchus contortus (Dalzell et. al. 2011)
Pristionchus pacificus (Dalzell et. al. 2011)

TSN-1 Sequences

AAH17180.3 (NCBI)
AA04505.1 (NCBI)
NP_612021.1 (NCBI)
Smp_246840 (Wormbase Parasite)
Smp_246850 (Wormbase Parasite)
CE02626 (Wormbase)
BM43562 TSN-1 (Wormbase)
CBP01117 (Wormbase)
JA55899 (Wormbase)
RP14297 (Wormbase)
OVP12598 (Wormbase)
PP14068 (Wormbase)
Caenorhabditis brenneri (Dalzell et. al. 2011)
Meloidogyne hapla (Dalzell et. al. 2011)
Meloidogyne incognita (Dalzell et. al. 2011)
Oesophagostomum dentatum (Dalzell et. al. 2011)
Trichinella spiralis (Dalzell et. al. 2011)

VIG-1 Sequences

NP_523572.1 (NCBI)
CE11254 (Wormbase)
BM36903 (Wormbase)
CN15739 (Wormbase)
CBP07521 (Wormbase)
JA11839 (Wormbase)
RP37119 (Wormbase)
OVP08750 (Wormbase)
PP06425 (Wormbase)
Ancylostoma caninum (Dalzell et. al. 2011)
Ascaris suum (Dalzell et. al. 2011)
Oesophagostomum dentatum (Dalzell et. al. 2011)

ADAR Sequences

P55265.4 (NCBI)
XP_015318055.1 (NCBI)
NP_569940.2 (NCBI)
CE32459 (Wormbase)
CBP93236 (Wormbase)
RP07872 (Wormbase)
Ancylostoma caninum (Dalzell et. al. 2011)
Ascaris suum (Dalzell et. al. 2011)
Brugia malayi (Dalzell et. al. 2011)
Caenorhabditis brenneri (Dalzell et. al. 2011)
Ancylostoma caninum (Dalzell et. al. 2011)
Ascaris suum (Dalzell et. al. 2011)
Brugia malayi (Dalzell et. al. 2011)
Caenorhabditis brenneri (Dalzell et. al. 2011)
Haemonchus contortus (Dalzell et. al. 2011)
Oesophagostomum dentatum (Dalzell et. al. 2011)

ADAR-2 Sequences

P78563.1 (NCBI)
XP_005202097.1 (NCBI)
AAF63702.1 (NCBI)
CE25120 (Wormbase)
CN05541 (Wormbase)
CBP4602 (Wormbase)
JA47475 (Wormbase)
RP33924 (Wormbase)
PP29926 (Wormbase)
Ancylostoma caninum (Dalzell et. al. 2011)
Ascaris suum (Dalzell et. al. 2011)
Haemonchus contortus (Dalzell et. al. 2011)
Meloidogyne hapla (Dalzell et. al. 2011)
Oesophagostomum dentatum (Dalzell et. al. 2011)

ER1 Sequences

NP_699163.2 (NCBI)
NP_001033281.1 (NCBI)
XP_011426949.1 (NCBI)
CE17214 (Wormbase)
Smp_0057290 (Wormbase Parasite)
Smp_051420.1 (Wormbase Parasite)
Smp_157760 (Wormbase Parasite)
Ascaris suum (Dalzell et. al. 2011)
BM22822 (Wormbase)
Caenorhabditis brenneri (Dalzell et. al. 2011)
CBP17865 (Wormbase)
JA63029 (Wormbase)
RP30882 (Wormbase)
Haemonchus contortus (Dalzell et. al. 2011)
Meloidogyne hapla (Dalzell et. al. 2011)
Meloidogyne incognita (Dalzell et. al. 2011)
Oesophagostomum dentatum (Dalzell et. al. 2011)
PP-PP45833 Pristionchus pacificus (Dalzell et. al. 2011)

Trichinella spiralis (Dalzell et. al. 2011)

ERI-3 Sequences

CE32072 (Wormbase)
CBP45144 (Wormbase)
RP30649 (Wormbase)

ERIS Sequences

CE39434 (Wormbase)
CN20399 (Wormbase)
CBP45144 (Wormbase)
JAG1238 (Wormbase)
RP23017 (Wormbase)

ERIG Sequences

CE08658 (Wormbase)
Caenorhabditis brenneri (Dalzell et. al. 2011)
Caenorhabditis briggsae (Dalzell et. al. 2011)
Caenorhabditis japonica (Dalzell et. al. 2011)
Caenorhabditis remanei (Dalzell et. al. 2011)

ERIT Sequences

CE36108 (Wormbase)
Caenorhabditis brenneri (Dalzell et. al. 2011)
Caenorhabditis briggsae (Dalzell et. al. 2011)
Caenorhabditis japonica (Dalzell et. al. 2011)
Caenorhabditis remanei (Dalzell et. al. 2011)

lin-15b Sequences

CE15381 (Wormbase)
CBP44496 (Wormbase)
JA63349 (Wormbase)
Caenorhabditis brenneri (Dalzell et. al. 2011)
Caenorhabditis remanei (Dalzell et. al. 2011)

XRN1 Sequences

AAH59314.1 (NCBI)
XP_005102950.1 (NCBI)
NP_523408.2 (NCBI)
Smp_011444428.1 (NCBI)
Smp_140420 (Wormbase Parasite)
Sjp_0051080 (Wormbase Parasite)
CE42924 (Wormbase)
BM46312 (Wormbase)
CN18416 (Wormbase)
CBP41142 (Wormbase)
JA65206 (Wormbase)
RP23844 (Wormbase)
OVP05624 (Wormbase)
BP43664 (Wormbase)
Ascaris suum (Dalzell et. al. 2011)
Haemonchus contortus (Dalzell et. al. 2011)
Oesophagostomum dentatum (Dalzell et. al. 2011)
Trichinella spiralis (Dalzell et. al. 2011)

XRN2 Sequences

NP_001304889.1 (NCBI)
XP_010809536.1 (NCBI)
NP_609082.1 (NCBI)
XP_011451785.1 (NCBI)
Smp_133300 (Wormbase Parasite)
Sjp_0075730 (Wormbase Parasite)
CE42702 (Wormbase)
CBP30614 (Wormbase)
JA59509 (Wormbase)
RP35486 (Wormbase)
OVP13756 (Wormbase)
PP42135 (Wormbase)
Ancylostoma caninum (Dalzell et. al. 2011)
Ascaris suum (Dalzell et. al. 2011)
Brugia malayi (Dalzell et. al. 2011)
Caenorhabditis brenneri (Dalzell et. al. 2011)
Haemonchus contortus (Dalzell et. al. 2011)
Meloidogyne hapla (Dalzell et. al. 2011)
Meloidogyne incognita (Dalzell et. al. 2011)
Oesophagostomum dentatum (Dalzell et. al. 2011)
Trichinella spiralis (Dalzell et. al. 2011)

RSD-2 Sequences

CE40653 (Wormbase)
CBP41205 (Wormbase)
JA45044 (Wormbase)
Caenorhabditis remanei (Dalzell et. al. 2011)

RSD-3 Sequences

Q14677.1 (NCBI)

NP_001098887.1 (NCBI)
EK32147.1 (NCBI)
Sjp_0064420 (Wormbase Parasite)
CE03062 (Wormbase)
BM19311 (Wormbase)
CBP34856 (Wormbase)
JA44423 (Wormbase)
RP30343 (Wormbase)
OVP04093 (Wormbase)
PP29990 (Wormbase)
Ancylostoma caninum (Dalzell et. al. 2011)
Ascaris suum (Dalzell et. al. 2011)
Caenorhabditis brenneri (Dalzell et. al. 2011)
Haemonchus contortus (Dalzell et. al. 2011)
Meloidogyne hapla (Dalzell et. al. 2011)
Meloidogyne incognita (Dalzell et. al. 2011)
Oesophagostomum dentatum (Dalzell et. al. 2011)
Trichinella spiralis (Dalzell et. al. 2011)

RSD-6 Sequences

BSMCY1.1 (NCBI)
XP_015329037.1 (NCBI)
CE32384 (Wormbase)
CN04313 (Wormbase)
CBP14864 (Wormbase)
JA42867 (Wormbase)
RP29589 (Wormbase)
Pristionchus pacificus (Dalzell et. al. 2011)

SID-1 Sequences

AA17723.1 (NCBI)
XP_005203130.1 (NCBI)
Smp_152020 (Wormbase Parasite)
Sjp_0101810/Sjp_0067130 (Wormbase Parasite)
CE03031 SID1 (Wormbase)
BM45574 SID1 (Wormbase)
CN02065 (Wormbase)
CBP2114 (Wormbase)
JA60059 (Wormbase)
RP37195 (Wormbase)
OVP05450 (Wormbase)
Haemonchus contortus (Dalzell et. al. 2011)
Oesophagostomum dentatum (Dalzell et. al. 2011)

SID-2 Sequences

CE33908 (Wormbase)
CN00799 (Wormbase)
CBP19178 (Wormbase)
JA02006 (Wormbase)
RP38789 (Wormbase)
EGO-1 Sequences
CE27140 (Wormbase)
CN2005 (Wormbase)
CBP37945 (Wormbase)
JA11806 (Wormbase)
Ascaris suum (Dalzell et. al. 2011)
Brugia malayi (Dalzell et. al. 2011)
Meloidogyne hapla (Dalzell et. al. 2011)
Meloidogyne incognita (Dalzell et. al. 2011)
Oesophagostomum dentatum (Dalzell et. al. 2011)

MUT-7 Sequences

NP_060290.3 (NCBI)
XP_015321064.1 (NCBI)
XP_011434475.1 (NCBI)
NP_610094.1 (NCBI)
CE00370 (Wormbase)
CN38432 (Wormbase)
CBP46637 (Wormbase)
JA59525 (Wormbase)
RP29673 (Wormbase)
Ancylostoma caninum (Dalzell et. al. 2011)
Ascaris suum (Dalzell et. al. 2011)
Brugia malayi (Dalzell et. al. 2011)
Haemonchus contortus (Dalzell et. al. 2011)
Meloidogyne hapla (Dalzell et. al. 2011)
Oesophagostomum dentatum (Dalzell et. al. 2011)
Pristionchus pacificus (Dalzell et. al. 2011)
Trichinella spiralis (Dalzell et. al. 2011)

RDE-2 Sequences

CE37005 (Wormbase)
CBP43782 (Wormbase)
JA47859 (Wormbase)
RP28943 (Wormbase)
Caenorhabditis brenneri (Dalzell et. al. 2011)

SMG-2 Sequences

Q29902.2 (NCBI)
DAA28309.1 (NCBI)
EK321342.1 (NCBI)
NP_572767.1 (NCBI)
Sjp_0010660 (Wormbase Parasite)
Smp_061590.1 (Wormbase Parasite)
CE23867 (Wormbase)
BM43052 (Wormbase)
CN24885 (Wormbase)
CBP02073 (Wormbase)
JAG6560 (Wormbase)
RP46543 (Wormbase)
OVP14320 (Wormbase)
PP44786 (Wormbase)
Ancylostoma caninum (Dalzell et. al. 2011)
Ascaris suum (Dalzell et. al. 2011)
Haemonchus contortus (Dalzell et. al. 2011)
Meloidogyne hapla (Dalzell et. al. 2011)
Meloidogyne incognita (Dalzell et. al. 2011)
Oesophagostomum dentatum (Dalzell et. al. 2011)
Trichinella spiralis (Dalzell et. al. 2011)

SMG-5 Sequences

BAC53623.1 (NCBI)
NP_001077448.1 (NCBI)
Sjp_0091360 (Wormbase Parasite)
Smp_169530 (Wormbase Parasite)
CE25134 (Wormbase)
CN05132 (Wormbase)
CBP14967 (Wormbase)
JA21381 (Wormbase)
RP21962 (Wormbase)

SMG-6 Sequences

Q86158.2 (NCBI)
NP_001182756.1 (NCBI)
EKC33850.1 (NCBI)
Smp_131650 (Wormbase Parasite)
CE32984 (Wormbase)
BM34749 (Wormbase)
CBP4366 (Wormbase)
JA01269 (Wormbase)
OVP04827 (Wormbase)
Ancylostoma caninum (Dalzell et. al. 2011)
Ascaris suum (Dalzell et. al. 2011)
Caenorhabditis brenneri (Dalzell et. al. 2011)
VLRSLCULTDRLTKAGNPFKRGINAKWVG
Haemonchus contortus (Dalzell et. al. 2011)
Meloidogyne hapla (Dalzell et. al. 2011)
Meloidogyne incognita (Dalzell et. al. 2011)
Oesophagostomum dentatum (Dalzell et. al. 2011)

Pristionchus pacificus (Dalzell et. al 2011)
Trichinella spiralis (Dalzell et. al 2011)

CID-1 Sequences

NP_001009881.1 (NCBI)
XP_010801816.1 (NCBI)
XP_011517316.1 (NCBI)
XP_010806440.1 (NCBI)
CE02015 (Wormbase)
CN15335 (Wormbase)
CBP20090 (Wormbase)
JAS3700 (Wormbase)
RP42885 (Wormbase)
OVPO3099 (Wormbase)

EKL-1 Sequences

CE05687 (Wormbase)
BM41207 (Wormbase)
CBP37258 (Wormbase)
JAG60996 (Wormbase)
RP28013 (Wormbase)
PP41586 (Wormbase)
Ancylostoma caninum (Dalzell et. al 2011)
Caenorhabditis brenneri (Dalzell et. al 2011)
Haemonchus contortus (Dalzell et. al 2011)
Meloidogyne hapla (Dalzell et. al 2011)
Meloidogyne incognita (Dalzell et. al 2011)
Oesophagostomum dentatum (Dalzell et. al 2011)

EKL-4 Sequences

NP_001029195.1 (NCBI)
EKX31208.1 (NCBI)
Smp_0002160 (Wormbase Parasite)
Sjp_0000610 (Wormbase Parasite)
CE29836 (Wormbase)
BM20893 (Wormbase)
CBP08051 (Wormbase)
JAS39535 (Wormbase)
RP44403 (Wormbase)
OVPO5333 (Wormbase)
PP48715 (Wormbase)
Ascaris suum (Dalzell et. al 2011)
Caenorhabditis brenneri (Dalzell et. al 2011)
Haemonchus contortus (Dalzell et. al 2011)
Meloidogyne hapla (Dalzell et. al 2011)
Meloidogyne incognita (Dalzell et. al 2011)

EKL-5 Sequences

CE44333 (Wormbase)
CN17207 (Wormbase)
JAS3094 (Wormbase)
RP10702 (Wormbase)
Caenorhabditis briggsae (Dalzell et. al 2011)

EKL-6 Sequences

NP_078838.1 (NCBI)

XP_019834464.1 (NCBI)
NP_609922.2 (NCBI)
CE51593 (Wormbase)
BM41419 (Wormbase)
CN05593 (Wormbase)
JAS59875 (Wormbase)
RP31352 (Wormbase)
OVPI0637 (Wormbase)
PP34905 (Wormbase)

Ancylostoma caninum (Dalzell et. al 2011)
Caenorhabditis briggsae (Dalzell et. al 2011)
Haemonchus contortus (Dalzell et. al 2011)

GFL-1 Sequences

NP_006521.1 (NCBI)
NP_001069833.1 (NCBI)
XP_011427964.1 (NCBI)
AAFS2462.2 (NCBI)
Smp_070760 (Wormbase Parasite)
Sjp_0068080 (Wormbase Parasite)
CE12388 (Wormbase)
BM41536 (Wormbase)
CBP15531 (Wormbase)
JAL12698 (Wormbase)
RP19851 (Wormbase)
OVPI1512 (Wormbase)
Ancylostoma caninum (Dalzell et. al 2011)
Ascaris suum (Dalzell et. al 2011)
Haemonchus contortus (Dalzell et. al 2011)
Meloidogyne incognita (Dalzell et. al 2011)
Oesophagostomum dentatum (Dalzell et. al 2011)
Trichinella spiralis (Dalzell et. al 2011)

MES-2 Sequences

CAA64955.1 (NCBI)
NP_001095621.1 (NCBI)
NP_524021.2 (NCBI)
Smp_164650 (Wormbase Parasite)
Sjp_0072180 (Wormbase Parasite)
CE28067 (Wormbase)
BM29030 (Wormbase)
CN34880 (Wormbase)
CBP33838 (Wormbase)
JAG4095 (Wormbase)
OVPO9541 (Wormbase)
Ancylostoma caninum (Dalzell et. al 2011)
Ascaris suum (Dalzell et. al 2011)
Caenorhabditis remanei (Dalzell et. al 2011)
Haemonchus contortus (Dalzell et. al 2011)
Meloidogyne incognita (Dalzell et. al 2011)
Oesophagostomum dentatum (Dalzell et. al 2011)
Trichinella spiralis (Dalzell et. al 2011)

MES-3 Sequences

CE11046 (Wormbase)
CBP38928 (Wormbase)

JAG7604 (Wormbase)
RP41043 (Wormbase)
Caenorhabditis brenneri (Dalzell et. al 2011)

MES-4 Sequences

CE27781 (Wormbase)
BM24937 (Wormbase)
CBP07413 (Wormbase)
JAS5637 (Wormbase)
RP48484 (Wormbase)
OVPI4309 (Wormbase)
PP39245 MES-4 (Wormbase)

MES-6 Sequences

O75530.2 (NCBI)
Q35Z25.1 (NCBI)
Q26458.1 (NCBI)
Smp_165220 (Wormbase Parasite)
CE24796 (Wormbase)
BM42303 (Wormbase)
CN03316 (Wormbase)
CBP39565 (Wormbase)
JAQ2512 (Wormbase)
RP31594 (Wormbase)
OVPO5048 (Wormbase)
Ancylostoma caninum (Dalzell et. al 2011)
Ascaris suum (Dalzell et. al 2011)
Haemonchus contortus (Dalzell et. al 2011)
Meloidogyne hapla (Dalzell et. al 2011)
Oesophagostomum dentatum (Dalzell et. al 2011)

MUT-2 Sequences

CE11740 (Wormbase)
CN15799 (Wormbase)
CBP17632 (Wormbase)
JAS8183 (Wormbase)
RP24216 (Wormbase)
PP42162 (Wormbase)
Haemonchus contortus (Dalzell et. al 2011)

MUT-14 Sequences

CE06825 (Wormbase)
CE28975 (Wormbase)
CBP16557 (Wormbase)
RP31627 (Wormbase)
JAG5879 (Wormbase)

MUT-15 Sequences

CE23936 (Wormbase)
CBP46299 (Wormbase)
JAS7376 (Wormbase)
RP44547 (Wormbase)

MUT-16 Sequences

CE40346 (Wormbase)
CBP44329 (Wormbase)

JAG3728 (Wormbase)
RP48608 (Wormbase)
Caenorhabditis brenneri (Dalzell et. al 2011)

RHA-1 Sequences

NP_001348.2 (NCBI)
NP_776461.1 (NCBI)
NP_476641.1 (NCBI)
CE39177 (Wormbase)
BM42381 (Wormbase)
CN00840 (Wormbase)
CBP39763 (Wormbase)
JAG0844 (Wormbase)
RP26542 (Wormbase)
Ascaris suum (Dalzell et. al 2011)
Haemonchus contortus (Dalzell et. al 2011)
Meloidogyne hapla (Dalzell et. al 2011)
Meloidogyne incognita (Dalzell et. al 2011)
Trichinella spiralis (Dalzell et. al 2011)

RRF-3 Sequences

CE45624 (Wormbase)
BM43371 (Wormbase)
CN14535 (Wormbase)
CBP00181 (Wormbase)
JAG2852 (Wormbase)
RP37819 (Wormbase)
OVPI2943 (Wormbase)
PP41583 (Wormbase)
Ancylostoma caninum (Dalzell et. al 2011)
Ascaris suum (Dalzell et. al 2011)
Haemonchus contortus (Dalzell et. al 2011)
Trichinella spiralis (Dalzell et. al 2011)

ZFP-1 Sequences

AA147519.1 (NCBI)
XP_005194335.3 (NCBI)
EKX26968.1 (NCBI)
CE25003 (Wormbase)
BM37915 (Wormbase)
CN00487 (Wormbase)
CBP30359 (Wormbase)
JAG6345 (Wormbase)
RP38280 (Wormbase)
PP37542 (Wormbase)
Ascaris suum (Dalzell et. al 2011)
Brugia malayi (Dalzell et. al 2011)
Haemonchus contortus (Dalzell et. al 2011)
Meloidogyne hapla (Dalzell et. al 2011)

Supplementary File 2

fhcam2 and *F. hepatica* cathepsin L (*fhcatL*) dsRNAs were generated as described in main methods (primers listed in Supplementary Table 2). Glass pipettes were pulled using a PC-10 Narishige Puller (3½" glass pipettes; 7 mm drop; Heater 1 setting: 59.1; Heater 2 setting: 58.1) and broken approximately 1 mm from the end to enable liquid to be drawn up. A Drummond "Nanoject" XV Microinjector (Drummond Scientific Company) was used to inject adult liver fluke (those that had regurgitated gut contents) with 18.2 nl of dsRNA (100 ng/μl) or RPMI at seven sites around the worm. Adult worms were maintained in RPMI+ (RPMI with gentamicin (Sigma-Aldrich) diluted 1 in 100) for 18 h before transfer to CS20 (20% chicken serum in RPMI+). Those adults that were soaked in dsRNA were soaked in 5 ml of 100 ng/μl dsRNA for 18 h prior to transfer to CS20. All adults were maintained in 30 ml media for 48 h post dsRNA exposure before being snap frozen for further analysis. All adults were maintained in groups of three.

Adult liver fluke RNA was extracted by lysing single adults in 1 ml of TRIzol® (ThermoFisher Scientific) and following kit instructions. RNA pellets were suspended in 50 μl nuclease free water with 2000 ng of nucleic acid (measured on Nanodrop 2000) DNase treated prior to reverse transcription as described in main methods. qPCRs were run on diluted cDNA (1:10) and data treated in the same way as described in main methods. Protein was extracted using RIPA buffer, as described in McVeigh et al. (2014), although 1 ml extraction buffer was used. Actin (20–33, Sigma Aldrich) was used as control band with analysis carried out as described in main methods.